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Adaptive Mechanisms for Aquatic Existence in Freshwater Turtles

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ADAPTIVE MECHANISMS FOR
AQUATIC EXISTENCE IN
FRESHWATER TURTLES

Presented by

Oscar Gloor

•Spring, 1977

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I. INTRODUCTION

Naturalists have often marveled at the ability of some air-breathing vertebrates to remain underwater for long periods of time (that is, "long" from man's reckoning). Seals, penguins, porpoises, and whales are all noted for their ability to use oxygen stored in various ways to permit them to "stay under" for many minutes.

The return of vertebrates, which evolved on land, to an aquatic existence has been a much discussed subject from an evolutionary standpoint. That there are many advantages to life underwater is easily seen simply by the fact that, according to fossil records, terrestrial life arose from the sea. Water is such a necessary component in protoplasm that it took eons of evolutionary change to produce organisms capable of surviving outside of it. To the vertebrate amniote, though, having gained physiological independence from an aquatic existence, competition from other land-dwellers and the relatively stable and rich environments found underwater make a return to their primeval dwelling place a viable alternative.

However, after millions of years of adapting to life on land, many of the features "taken for granted" by aquatic creatures had been lost. These animals had developed legs and had lost fins. The streamlined shape necessary for aquatic mobility had in most cases been drastically modified. Most importantly, they had developed a dependence ^{ON} atmospheric oxygen. The surfaces for gas exchange had been modified to facilitate this dependence, and the

relative richness of the oxygen supply in the air allowed metabolic rates to be raised, even to the point of producing what we know as warm-blooded animals.

Thus, the animal attempting to ~~readjust~~^{readjust} to an aquatic existence encounters many adaptive obstacles. However, many species of amniotes (reptiles, birds, mammals) have made this return in varying degrees. Perhaps of these three classes, the reptiles have more aquatic or semi-aquatic species than either of the other two groups. Of the reptiles, the turtles (order Chelonia) are the most aquatic in habits. Many are wholly aquatic, and most of the group are at least semi-aquatic.

It is the purpose of this paper to examine some of the recent work done on one phase of the turtles' "retrogressive evolution"--their adaptations to life underwater without gills.

Turtles breathe air by means of lungs, as do other amniotes. ~~But~~ What permits turtles to stay underwater for hours at a time? How does a turtle survive buried in the mud at the bottom of a pond for the duration of the winter months? Even more intriguing is the ability of turtles injected with cyanide (which stops oxidative processes in the mitochondria) to survive for twenty hours.

Recent (and some not-so-recent) work has examined some of the adaptive mechanisms of some turtles and has illuminated many of the anatomical and physiological features which permit chelonians to be so well adapted to hypoxic and anoxic environments.

II. RECENT WORK

Most of the research done in the area can be summarized by classifying the various mechanisms which were, for the most part, studied independently of the others. Unfortunately, most of the researchers centered their studies on one aspect of one species' adaptive mechanisms. Many workers viewed the other adaptive mechanisms (that is, those which they were not studying) as insignificant in the total adaptive mechanism, and often failed to even attempt to correlate their findings with those of others. A truly comprehensive study of this field has yet to be performed.

The three main areas of research concerning aquatic respiratory mechanisms are given below:

- (1) Dermal and Buccopharyngeal Respiration
- (2) Anaerobiosis
- (3) Bradycardia and Vasoconstriction

Dermal and Buccopharyngeal respiration were first studied by [Gage and Gage] (1886) in a classic study of the soft-shelled turtle (*Trionyx* spp.). In this study, it was shown that these turtles actually use oxygen from the water by gas exchange across the highly vascularized areas of the skin and the mucous membranes of the pharynx. This study was the first to identify the rhythmic movements of the hyoid apparatus as being respiratory in function. Although questionable in its relative importance, the alternate expansion and contraction of the pharynx, with its resultant inspiration and expiration of water, has been studied widely in attempts to establish the degree to which this mechanism enhances underwater survival.

In 1962 [Daniel Belkin] injected musk turtles (Sternotherus minor) iodoacetic acid (sublethal doses), which prevents glycolysis, and found that injected turtles only survived an average of 0.32 hours in an anaerobic environment, while uninjected turtles under the same conditions survived for an average of 12.2 hours. His conclusion that glycolysis and other sources of anaerobic energy production were the primary reasons for prolonged survival of turtles initiated a flurry of interest in the turtle's metabolic adaptations, most notably, the utilization of glycolysis as an energy source.

Belkin (1964) also performed a study on heart rates in turtles and found that turtles reduce heart rates up to 66% during voluntary ^{DIVES} bradycardia occurred within one or two beats after the dive began. This reaction, apparently mediated by higher centers of the central nervous system, seems to reflect a significantly lowered metabolic rate during dives, which would certainly be advantageous in the conservation of existing oxygen supplies.

Wide agreement exists that the above three phenomena occur and help to facilitate prolonged dives, but there still exist controversies surrounding the relative importance of each. Studies on some turtles seem to indicate that buccopharyngeal respiration is insignificant in survival times. Studies on other turtles, though, show buccopharyngeal respiration to be an efficient method of obtaining significant amounts of oxygen.

The chief difficulty in all of these studies is the wide variability that exists among the species of fresh water turtles. In some cases invalid generalizations were drawn from studies of one species. Although the role of anaerobiosis in turtle diving

is now a well-established fact, the importance of buccopharyngeal respiration and bradycardia remains to be determined for most of the species. These two characteristics (especially the former), as will be seen below, vary significantly from species to species.

ANAEROBIOSIS

Two years after Belkin's iodoacetate experiment showing the importance of glycolysis, Robin, et al (1964) undertook a fairly detailed study of the phenomenon, using blood sampling methods to determine some of the metabolic effects on the blood under varying environmental conditions (diving, N_2 inhalation, cyanide injection). The results bore out Belkin's conclusion that anaerobiosis was a facilitative mechanism. As would be expected, blood pH fell. O_2 levels also fell to very low levels (except in the case of $NaCN$ injections in which case, of course, oxygen was present, but was not utilized due to the inhibitory effect of CN on oxidative processes). A fall in blood HCO_3^- concentration in the diving phase indicated that the acidosis produced was not only a product of CO_2 retention, but was also due to the accumulation of lactic acid, a by-product of glycolysis.

One point made in the above study was that results for diving (in water equilibrated with 100% O_2) were essentially similar to those obtained in the group exposed to pure N_2 atmospheres. This fact, according to the authors, seems to discount the importance of any extraction of oxygen through buccopharyngeal or dermal respiration in this species. However, there is a danger in applying this assumption to any other species, as will be seen. This work was

done on turtles of the genus Pseudemys, a group of turtles known as "sliders" and "cooters" commonly sold by scientific supply companies.

A later study by Clark and Miller (1973) used tissue assays on blood, brain, heart, and liver samples taken after varying intervals of anaerobiosis. They found that there was a progressive fall in liver and heart glycogen and a rise in blood glucose during anaerobiosis. While most others had suggested that the production of excess lactic acid and its deleterious effect on tissues was the limiting factor in the length of time in which the turtle could use anaerobiosis to sustain life, Clark and Miller contended that the limiting factor was the rapid depletion and exhaustion of ATP and creatine phosphate stores. They acknowledged that tissue acidity was a factor, but felt that the energetic inefficiency of glycolysis was primarily responsible for its limited value. This work also showed that significant decreases in brain glycogen did not occur. This is particularly important, since in most amniotes the limiting factor of anaerobic survival is the sensitivity of the central nervous system to hypoxia and anoxia. This is presumably due to the high dependence of the CNS on oxidative processes which enable the high energy bonds of ATP to be formed, so that the "sodium pump" can function effectively to carry impulses. However, Robin, et al (1964) suggest that since the "sodium pump" of turtle bladders can function anaerobically [Schlib et al (1962) and Bricker et al (1962)] then it might be possible for the turtle central nervous system to function likewise in some anomalous fashion. This, however, has not been verified.

Penney (1974), in a study similar to that performed by Clark and Miller (1973), found essentially the same results, also on Pseudemys. He also disproved a theory that lactic acid was buffered by large amounts of alkaline coelomic fluid present. Although Robin et al (1964) show that lactic acid can indeed penetrate into the coelom when (exogenously) applied, Penney found no such accumulation of lactic acid in the coelomic fluid following extended periods (20 hours) of anaerobiosis.

Belkin showed a mean tolerance of turtles (6 families, 25 species) for N_2 asphyxia of 14.75 hours at 22°C. Obviously, anaerobiosis is an important mechanism facilitating survival underwater where oxygen supplies are low. Although its universality can only be assumed, it is safe to say that turtles have a high tolerance for anoxia, and glycolysis is the main source of energy during periods of anoxia and hypoxia.

BRADYCARDIA

Although glycolysis is a viable energy source often used by tissues during stressful conditions, its energetic inefficiency in the production of ATP prevents its use for long periods of time. Anaerobic energy production by glycolysis occurs in the muscles of a sprinter, but as ATP is used up faster than it can be produced, the sprinter can only sprint for relatively short periods of time.

In the turtle, though, Belkin (1964) has discovered that the metabolism during dives is slowed down significantly, as evidenced by significant decreases in heart rate. By monitoring heart rates with continuous electrocardiograms, the turtles were monitored at

22°C during quiet, voluntary dives. He found that while underwater, heart rates were reduced significantly (up to 66%). When turtles *GRAY* surfaced by sticking the top of their nostrils above the surface, an immediate and radical increase in heart rate was observed. Wasserman and Mackenzie (1957) noted a similar phenomenon in seals. Other workers have observed bradycardia in fish when removed from water into air. Such observations suggest a common physiological response to asphyxia among vertebrates.

During Belkin's observations he noted that periods of submergence were, on the average, 50 times as long as the time spent at the surface. However, Belkin contends that the stored oxygen in the lungs and tissues could be substantial enough to provide enough oxygen to sustain life for 2 or 3 hours without physiological compensation. He notes some of his own unpublished data on kinosternid and testunid turtles which indicated that several species of these turtles have critical oxygen tensions well below 20 mm Hg. He suggests that this ability to extract oxygen at low partial pressures from inspired air must apply to oxygen stores as well, facilitating use of at least 90% of a turtle's oxygen stores.

Moreover, Belkin suggests that the low heart rates during diving are normal, and that the sudden increase when the turtle surfaces is a case of tachycardia in which the heart suddenly speeds up to satisfy the accumulated ventilatory demands as quickly as possible. As soon as these demands are met, the turtle dives again quickly and its slow rate of heart beat is resumed. Belkin feels that this may represent an adaptation to escape predation at the surface.

Belkin also notes that responses of other animals were not as immediate, but in all those cases, the test animals were subjected to forced diving. [Penney (1973) notes that bradycardia was not immediate in the Pseudemys he worked with] However, it should be noted that, ~~again~~, the animals in Penney's study were subjected to forced dives. [In his study of bradycardia, Belkin subjected turtles to nitrogen asphyxia and occlusion of the trachea, and found that neither produced the bradycardial response. However, injection with atropine, which eliminates the influence of the cardiac vagus nerve, caused the high heart rate seen at surfacing to continue throughout dives,] although behavior was otherwise normal. Therefore, one may conclude that the stimulus for bradycardia is neurogenic, rather than a product of chemical conditions produced by hypoxia.

Thus, we see another facilitating factor in the turtle's ability to prolong its dives for 20 hours or more. A low oxygen requirement for oxidative processes and slow rate of metabolism-- governed by a slowing of the heart--are both advantageous for an animal spending much of its time, where (by terrestrial standards) little oxygen is available. Already, we can see that turtle physiology differs markedly from the patterns seen in other vertebrates.

BUCCOPHARYNGEAL AND DERMAL RESPIRATION

After Gage and Gage (1886) first found that softshell turtles extract oxygen from water from the buccopharyngeal and dermal surfaces, years of speculation about the importance of this phenomenon (and, sadly, little experimentation) brought us into the 1960's

with little more knowledge about the subject than we had in 1900.

In addition to dermal and buccopharyngeal gas exchange, some scientists suggested that the cloaca, with its accessory bladders also served as an exchange surface. In 1960, William Dunson studied oxygen uptake of each of the three modes of gas exchange in the softshelled turtle (Trionyx). By various methods, he prevented each of the three modes of respiration from functioning two at a time, in various combinations, to observe the oxygen uptake of each mode exclusively. According to Dunson's measurements of ec of O₂ used per gram of turtle, pharyngeal respiration accounted for the bulk of the oxygen used. However, Dunson's methods leave themselves open to criticism and his results cannot be viewed as highly credible from the quantitative standpoint. His results, though, do indicate an appreciable amount of oxygen uptake, and seem to support the idea that such mechanisms do indeed function, at least in the case of the soft-shell turtle.

As pointed out before, the studies of anaerobiosis on Pseudemys (Clark and Miller, 1973; Penney, 1973; Belkin, 1963) all seem to discount any appreciable oxygen intake by these methods. In their metabolic studies, results were essentially identical for turtles diving in water and exposed to pure N₂ atmospheres. In both cases, little evidence of oxidative processes was seen in tissue and blood assays. These researchers felt that the importance of "aquatic respiration" was a myth and, if present, played only a minor role in sustaining life during prolonged dives. This seemed true, since turtles had been kept alive for up to 2 weeks at 16°-18°C (Robin et al, 1964) in an anaerobic environment.

In a detailed study of two turtles, Sternotherus minor and Pseudemys scripta, Belkin (1968) investigated oxygen uptake and survival times of these turtles under varying conditions. The three ^{BREATHING} conditions under which the test animals were placed were air, water (N₂ equilibrated), water (air equilibrated), and water (O₂ equilibrated). His results showed little oxygen uptake by Pseudemys in either the air or O₂ equilibrated water, which is consistent with the observations of other workers previously noted. However, in Sternotherus, O₂ uptake levels approached those recorded for air breathing in the experiment involving O₂ equilibrated water. (Diving turtles in this experiment had no access to the atmosphere.) Uptake levels for Sternotherus in air-equilibrated water were also 7 times larger than uptake levels for Pseudemys under the same conditions. These results tend to indicate that although little oxygen uptake from the water occurs in Pseudemys, appreciable amounts can be extracted by Sternotherus.

In his study of survival times of the turtles under these same conditions, Belkin's results were quite interesting. In the case of Pseudemys, survival rates for O₂ equilibrated water were only slightly higher than for N₂ equilibrated water, with air-equilibrated water falling in between these two. Although the Sternotherus in the N₂ equilibrated showed survival times close to that of Pseudemys, those in air equilibrated water survived up to 6 times longer (120 hours) than the corresponding Pseudemys group.

Truly remarkable, though, is the fact that the Sternotherus in the O₂ equilibrated water survived for 6 months without a single breath of atmospheric oxygen. After 6 months, the experiment was terminated with the turtles still alive. Belkin also noted that

when the oxygen supply was out off, some of the turtles did not even attempt to go to the surface for air, and, as a result, drowned. Those that did go to the surface reacted at first to air as if it was a noxious substance.

Although Belkin's experiments were under conditions which do not resemble the natural environment in which these turtles exist, it certainly proves that some turtles have the ability to extract oxygen from water through various body surfaces. Although this has been investigated in the few genera mentioned above, it is obvious that more comprehensive research must be done on the various genera to determine how widespread this ability is among the different species of aquatic turtles.

DISCUSSION

It is clear from the studies related above that the turtle's ability to prolong dives can be attributed to several unusual physiological characteristics. However, a clear picture of how these factors is not yet available. Much work obviously remains to be done.

From the works mentioned above, though, one can piece together a more coherent understanding of the processes. Anaerobiosis is clearly the main factor in prolonging dives. The author suggests this is a widely used mechanism among chelonians. Bradycardia probably is a similarly universal phenomenon among turtles. These two factors working together represent a basis for a model of chelonian physiological patterns. In nature, the turtle does not exist in a totally anaerobic environment, but has free access to the oxygen

he needs. The turtle probably uses both oxidative and anaerobic processes simultaneously in energy production, the dominance of either depending on the available supplies. When the turtle dives, he reduces his oxidative processes to a minimal rate and begins to depend more on glycolytic energy [except in the case of the brain, where oxidative processes continue at the expense of other tissues as shown by Clark and Miller (1973)]. Therefore, although the rate of glycolysis remains the same, oxidative processes are severely restricted. The degree to which oxidative processes continue are contingent on oxygen supplies available through stored oxygen, atmospheric oxygen, and oxygen obtained through aquatic respiration (buccopharyngeal and dermal).

It is interesting to note that turtles are regarded by some taxonomists as "living fossils" since there are very few anatomical differences between modern chelonians and the ancestral prototype. This primitive nature leads the author to suggest that such anomalous physiological patterns may not represent recent adaptations, but traits retained from eons past when oxygen was not as plentiful, and physiological patterns were radically different from those we are familiar with today.

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